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DNA phenotyping to find a natural enemy in Uzbekistan for California biotypes of *Salsola tragus* L.

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Abstract

Salsola tragus L. (Russian thistle, Chenopodiaceae), a weed of Central Asian origin, has two biotypes in California, type A and type B. The gall midge *Desertovellum stackelbergi* Mamaev (Diptera: Cecidomyiidae), which attacks *S. tragus* in Uzbekistan, is a candidate biological control agent for this weed in the United States. In a field test conducted in Uzbekistan with plants of the two biotypes of *S. tragus* from California, both biotypes were attacked by the insect, although type A was the preferred host. Accessions of *S. tragus* from Uzbekistan, Greece, and Ukraine were similar to the California type A when compared using RAPD and ISSR analyses, while California type B was distinct. Since both California biotypes were hosts to the gall midge, further studies on the biology and host specificity of the insect are justified. Genetic characterization of target weeds can provide information useful for the selection of natural enemies.

Keywords: Biological control; Biotypes; *Desertovellum stackelbergi*; Isoenzymes; ISSR; Molecular systematics; RAPD; Russian thistle; *Salsola*; Weed

1. Introduction

Knowledge of genetic variation in target organisms is essential for selection of effective biological control agents. Agents released for biological control of weeds must be highly specific to avoid damage to nontarget species while achieving control of targeted pests. Agents that are too specific, however, will attack only a subset of the targeted plants, resulting in incomplete control (Burdon et al., 1981; Hasan, 1985; Lym et al., 1996; Mayo and Roush, 1997; Sobhian and Andres, 1978). Biological agents that are able to attack all genetic types of the target weed have the greatest likelihood of exerting maximum control (Harris, 1985; Rosen, 1986).

Isoenzymes have been useful in determining genetic variation in weeds. DNA-based markers have great potential in detecting variation, in selecting probable areas of origin of invasive plants, and in matching biocontrol agents with problem plants (Nissen et al., 1995; Paltridge, 2000). For example, the rust fungus, *Puccinia chondrillina* Bub. & Syd., was introduced into Australia as a biological control agent for *Chondrilla juncea* L. but attacked only one morphological form of this invasive weed (Burdon et al., 1981), allowing other forms to increase their previously limited ranges. The other forms were genotypes with distinguishable isoenzyme complements (Burdon et al., 1980). This genetic information was used to find rust strains that were effective on the other genotypes (Hasan et al., 1995). Chloroplast DNA and nuclear RAPD markers have been used to ascertain genetic variability in *Euphorbia* species introduced into North America (Rowe et al., 1997). The relation between genotype in *Euphorbia esula* L. and the efficacy of a biocontrol agent was determined (Lym et al., 1996). Nevertheless, the European accessions have not been compared to those from North America using these techniques although their similarity is a key question

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(Gassmann and Schroeder, 1995). Other instances from the recent literature illustrate the use of DNA-based markers to determine probable sites of origin of invasive weeds (Scott et al., 1998a,b), to determine genetic correlates in an invasive grass in Australia, *Sporobolus indicus* (L.) R.Br., for susceptibility to a smut pathogen (Hetherington and Irwin, 1999), and to investigate genetic differences between species of horticultural importance and weedy relatives (Colosi and Schaal, 1997; Vaughn et al., 2001; Wetzel et al., 1999).

Isoenzyme and RAPD analyses of Russian thistle, *S. tragus* L. (= *S. kali* L. ssp. *tragus* (L.) Celak *sensu* European authors, but see Rilke (1999)) from California indicated the presence of two sympatric biotypes of this plant (Ryan and Ayres, 2000). Type A was identified as *S. tragus*, as known in Europe and Asia with a chromosome number of $2N = 36$. The taxonomic status of type B, with a chromosome number of $2N = 18$, remains unknown and it continues to be included within *S. tragus*. Candidate biocontrol organisms from Uzbekistan, near the center of origin for several species of *Salsola* (Pyankov et al., 2001; Rilke, 1999) have been screened as potential antagonists of *S. tragus* in the United States but, to be successful, potential agents should attack both biotypes of this weed. The gall midge *Desertovellum stackelbergi* Mamaev (Diptera: Cecidomyiidae) has been selected as potentially effective on *S. tragus* (Sobhian and Khamraev, unpublished). This gall midge, reported only from the former USSR, produces several generations per year. It overwinters as mature larvae in dry galls on old plants (O. Kovalev, St. Petersburg, Russia, personal communication). In order to compare differential susceptibility of the two California biotypes of *S. tragus* to the gall midge, these plants were exposed to the gall midge in its native range in Uzbekistan. DNA analyses were used to determine the similarity of *S. tragus* from Uzbekistan to the California types A and B and other accessions.

2. Material and methods

2.1. Field experiment in Uzbekistan

For field work in Uzbekistan, seeds of *S. tragus* type A were collected in Davis, California, during the winter of 1999, while those of *S. tragus* type B were collected from a population near the USDA ARS Horticultural Crops Research Laboratory, Fresno, California. During the previous growing season, plants from these locations were analyzed for aspartate aminotransferase and 6-phosphogluconate dehydrogenase (Ryan and Ayres, 2000) and biotype was assigned according to the patterns of isoenzymic activity, as well as morphological criteria, for instance, time of flowering and fruit set (Ryan and Ayres, 2000; Ryan et al., 2002). Identification of type was also made on a morphological basis when the seeds were collected.

The seeds of *S. tragus* type A and B from California were taken to Ultarma, Uzbekistan, and germinated on 3 April 2000, the beginning of the growing period for *S. tragus* in this region. Germinated seeds, 40 of each type, were planted in small peat pots within larger pots on April 5 and allowed to grow until transplanted to a field site near Ultarma in mid-May. The test plants were planted in a large, otherwise undisturbed field with local *S. tragus*, which carried populations of the gall midge. Experimental plants were grown in rows at an inter-row distance of 50 cm and an inter-plant distance of 30 cm. Local constraints dictated a cryptic field set-up with no labels or colored containers to attract attention. Infestation of the test plants with the gall midge was allowed to occur naturally, with no manipulation of the insects by personnel involved in the experiment. Test plants were watered as needed and checked for gall formation on 25 June and 16 July 2000. The number of plants attacked and the number of galls per plant were recorded. All the test plants of US origin were collected on 16 July and burned to prevent their further development and seed formation.

Because of the low level of infestation on *S. tragus* type B, several dried stems from each of the six plants of this type infested with galls were examined in Fresno using RAPD and inter-simple sequence repeat (ISSR) analyses to verify their identity.

2.2. Sources of other Salsola accessions used in DNA analyses

To analyze variation in *S. tragus* in Uzbekistan and determine the relation to plants growing in other regions of Eurasia, accessions were obtained from Greece, Ukraine, California, and Uzbekistan. Uzbek accessions were collected near Ultarma in August, 2001, at three sites approximately 1 km from the test site and one site (D) 15 km from the test site. The locations of these sites and the field plot are given in Table 1. At each site, eight plants were collected at 10-m intervals. Accessions of *S. tragus* types A and B were obtained from plants grown in a common garden (Summer 2000) at the California Department of Food and Agriculture, Sacramento, California. The original collection sites of the common garden plants are shown in Table 1. A specimen from Kozani, Greece (Table 1) was provided by J. Kashefi, USDA ARS European Biological Control Laboratory, Thessaloniki, Greece. Specimens from Ukraine were collected in September 2000 near Kiev and in Kherson Province, southern Ukraine at locations indicated in Table 1 by Dr. S. Mosyakin and colleagues, National Academy of Sciences, Kiev, Ukraine. Two specimens of *Salsola paulsenii* Litv. spinose form, used as an out-group, were also from the common garden in

Table 1
Site of origin for *S. tragus* and *S. paulsenii* used in this study

Abbreviation	Type	Original collection site	Lateral	Longitudinal
CA-A-Coalinga	A	Coalinga	36°9.18′N	120°21.23′W
CA-A-Fresno	A	Site 1, Fresno	36°48.90′N	119°51.50′W
CA-A-Davis	A	Davis	38°32.13′N	121°44.08′W
CA-B-Santa Nella	B	Santa Nella	37°4.26′N	121°1.23′W
CA-B-Fresno	B	Horticultural Crops Research Laboratory, Fresno	36°43.28′N	119°44.08′W
CA-B-San Diego	B	San Diego	32°46.06′N	117°9.18′W
Site A		Ultarma, Uzbekistan	40°26.85′N	71°5.33′E
Site B		Ultarma, Uzbekistan	40°28.45′N	71°5.33′E
Site C		Ultarma, Uzbekistan	40°30.13′N	71°4.23′E
Site D		Ultarma, Uzbekistan	40°54.15′N	70°45.57′E
Field experiment site		Ultarma, Uzbekistan	40°26.22′ N	71°4.83′E
Greece		Near Kozani	41°16.7′N	21°53.3′E
Ukraine (Ukr-1–3)		In Kiev	50°26.0′N	30°31.0′E
Ukraine (Ukr-4)		Novotroitskiy, Kherson province	47°21.0′N	33°41.0′E
Ukraine (Ukr-5)		Tsyurupin'sk, Kherson province	46°37.0′N	32°43.0′E
Ukraine (Ukr-6)		Gladkovka, Kherson province	46°24.0′ N	32°36.0′E
S. paulsenii		Barstow, CA	34°53.8′N	117°2.6′W

Sacramento and grown from seed from a single plant collected in Barstow, California. A specimen of *Salsola vermiculata* L. was collected by W. Abel, USDA APHIS, in the Temblor Range, San Luis Obispo County, California. This was included in the amplifications but was not analyzed further due to the large genetic distance between *S. vermiculata* and the *S. kali* section of *Salsola.*

2.3. DNA amplification and analysis

DNA was isolated as described (Ryan and Ayres, 2000). All amplifications were carried out in a Perkin–Elmer GeneAmp 9600 thermocycler using AmpliTaq (Applied BioSystems, Foster City, CA) with the fastest ramping times for temperature transitions. RAPD amplifications were conducted with primers C18, G11, and G12 (Operon Technologies, Emeryville, CA)[1] using conditions described in Ryan and Ayres (2000) except that the concentration of $MgCl_2$ for primers G11 and G12 was 3 mM. Denaturation at 94 °C for 2 min was followed by 40 cycles with annealing at 35 °C for 30 s, elongation at 72 °C for 1 min, and denaturation at 94 °C for 30 s followed by annealing step and then a final elongation at 72 °C for 8 min. ISSR primers 852, 884, 888, and 890 were from the Protein Structure Laboratory, University of British Columbia, Vancouver, British Columbia, Canada. Amplifications were conducted using AmpliTaq Gold (Applied BioSystems, Foster City, CA). An initial 10-min activation at 94 °C was followed by 40 cycles of annealing at 58 °C (Primers 888 and 890) or 55 °C (Primer 852) for 30 s, elongation for 2 min at 72 °C, and denaturation at 94 °C for 15 s. The amplification was concluded with a 30-s annealing and an 8-min elongation at 72 °C.

Amplification products were electrophoresed on 1.5% NuSieve 3:1 agarose (BioWhittaker, Rockland, ME) in 89 mM Tris with 89 mM boric acid and 2 mM sodium EDTA, pH 8.0, stained with ethidium bromide, and visualized in a UV transilluminator. For each plant specimen, amplified bands were scored as present or absent. Similarities among individuals were calculated using the simple matching coefficient in NTSYS-pc version 2.01 (Rohlf, 1997). The unweighted pair-group method, arithmetic average (UPGMA) was used to examine the relationships among accessions, using the same program. Goodness of fit between the similarity matrix and the dendrogram (Mantel's Z statistic) was estimated using the same program.

2.4. Data analysis

For statistical analysis of insect infestation, each plant was considered a replicate. A Student's *t* test (SAS Institute, 1996) was used to assess the significance of differences in mean insect infestation.

3. Results

On 25 June, 38 plants of *S. tragus* type A and 39 plants of *S. tragus* type B survived in the field. On this

[1] Mention of a brand name or supplier does not constitute a guarantee or warranty by the US Department of Agriculture and is not an endorsement over other similar products.

Table 2
Gall production by the midge *Desertovellum stackelbergi* on *Salsola tragus* from California grown near Ultarma, Uzbekistan

	Number of galls per plant	
	June	July
	Mean ± SD	Mean ± SD
Type A ($N = 38$)	11.4 ± 18.0	58.7 ± 50.9
No. of plants with galls	26	38
Type B ($N = 39$)	0.0	0.6 ± 2.0
No. of plants with galls (Plant 1 excluded)	0	5
t value	3.895	7.035
df	74	74
P value	0.001	<0.0001

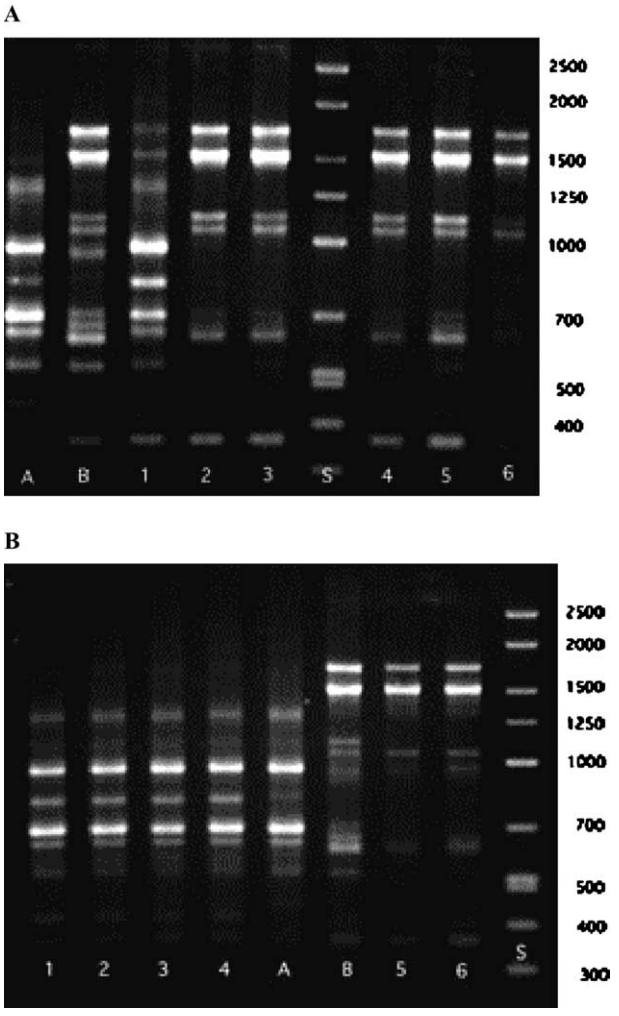

Fig. 1. Amplification products with primer Operon C18 of the six *S. tragus* type B plants infested with the gall midge. (A, B) Known *S. tragus* type A and type B from Fresno, CA; (1–6) specimens of *S. tragus* type B from Uzbekistan infested with the gall midge; (S) molecular weight standards with weights indicated in basepairs. (B) Amplification products with Operon primer C18 of single pieces of Plant 1 in A. (A, B) Known *S. tragus* type A and type B from Fresno, CA; (1–6) products from individual pieces of Uzbek Plant 1; 1–4 show patterns of amplified fragments characteristic of *S. tragus* type A, while 5 and 6 are patterns characteristic of type B; (S) molecular weight standards with weights indicated in basepairs.

date, 26 plants of the *S. tragus* type A (68.5%) and none of the *S. tragus* type B were infested by the gall midge, with one exception, described below. On July 16, all of the *S. tragus* type A and six of the *S. tragus* type B (15%) were infested. The mean number of galls per plant in June and July are shown in Table 2. At both observation times, *S. tragus* type A supported the larger number of galls per plant.

The six type B plants attacked by the gall midge were subjected to RAPD and ISSR analyses to verify their biotypes. Five of the type B plants produced amplified products characteristic of type B with the RAPD primer Operon C18 (Fig. 1A), precluding the possibility that midge infestation was due to accidental mixing of plants during handling and planting, i.e., that some type A plants had mistakenly been called type B. One specimen, designated Uzbek plant 1, however, had amplified products of both type A and type B (plant 1 in Fig. 1A). Subsequent analysis of individual stem pieces revealed the sample actually comprised pieces of plants of type A and type B (Fig. 1B). This plant also had a high rate of midge infestation, with 28 galls at the July determination compared to 1, 2, 3, 8, and 9 galls per plants for the other infested type B plants at the same time. Because of the anomalies in the DNA analysis, data from Uzbek plant 1 was not included in the analysis for Table 2.

For RAPD and ISSR analysis of *S. tragus* from Uzbekistan, Ukraine, Greece, and California, amplification with six primers produced 42 polymorphic bands. The molecular weights of the amplified products are given in Table 3. *S. tragus* from Uzbekistan showed a relatively low level of variation in these analyses (Fig. 2). In the UPGMA analysis, the 32 accessions from Uzbekistan clustered as 15 closely related phenotypes (Fig. 3). All the type A *S. tragus* from California formed a separate cluster that was closely associated with the material from Uzbekistan, Greece, and Ukraine. A type A piece from Uzbek plant 1 was most closely associated

Table 3
Polymorphic amplified products used to analyze similarities among *Salsola* accessions

OP-G11	OP-G12	OP-C18	855	888	890
640	450	580	730	480	440
860	620	680	880	690	570
1010	680	900	1340	770	580
1160	1050	1000	2000	930	600
1520	1960	1290		1350	680
1650		1770		1420	710
1950					750
					810
					890
					970
					1050
					1160
					1240
					1330

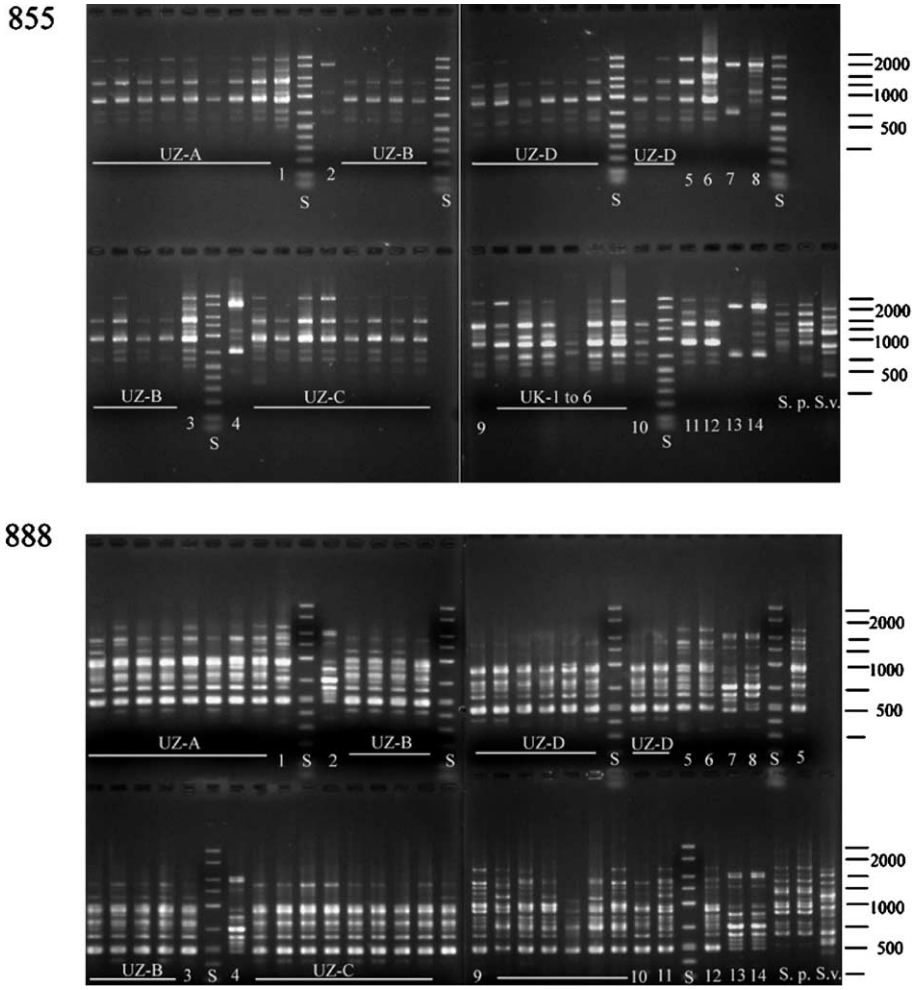

Fig. 2. Amplified products with ISSR primers 855 and 888. Molecular weight standards (S), indicated at the right side of each tier, were 2500, 2000, 1500, 1250, 1000, 700, and 500 bp. Specimens from four locations in Uzbekistan are indicated by lines within the figure. Other specimens were (1 and 3) type A, Davis; (2 and 4) type B, Santa Nella; (5 and 6) type A, Coalinga; (7 and 8) type B, San Diego; (9) type A piece of Uzbek Plant 1; (10) Greece (Table 1); (11 and 12) type A, Fresno; (13 and 14) type B, Fresno. Two specimens of *S. paulsenii* (S. p.) and one of *S. vermiculata* (S. v.) are also included.

with accessions from Uzbekistan, Ukraine, and Greece but not *S. tragus* from California, suggesting that it was of Uzbek origin. *S. paulsenii* clustered separately from *S. tragus*, and the type B plants were yet more distantly associated. *S. vermiculata* appeared as an outlier.

4. Discussion

The observation that type B *S. tragus* were colonized by the gall midge, even though the midge preferred type A, is of significance in considering the gall midge for use as a biocontrol agent. If one of the *Salsola* biotypes from California had been resistant, its priority ranking as a control agent would have been lowered and it might have been removed from further study since it would lack the necessary generality. Because the insect attacked both biotypes under the field conditions where there was a choice of hosts, we believe that it has potential as a biocontrol agent, although testing of its ability to attack other Chenopodiaceae and other plant species of concern needs to be completed.

The observation of biotype preference by the gall midge shows the value of understanding the genetic structure of target weed populations prior to testing of biological control agents. Previous studies have shown that *S. tragus* in California consists of at least two biotypes (Ryan and Ayres, 2000; Ryan et al., 2002). As a result, representatives of the two biotypes were included in the host specificity tests and differences in preference were identified. By testing representatives of both biotypes, the chances of selecting the most effective control agents are increased.

The analysis of the type B specimens from the field experiment illustrates another use of the molecular techniques. The analysis was prompted by the low rate of infestation of type B plants in the field. Five of the infested plants were clearly *S. tragus* type B according to

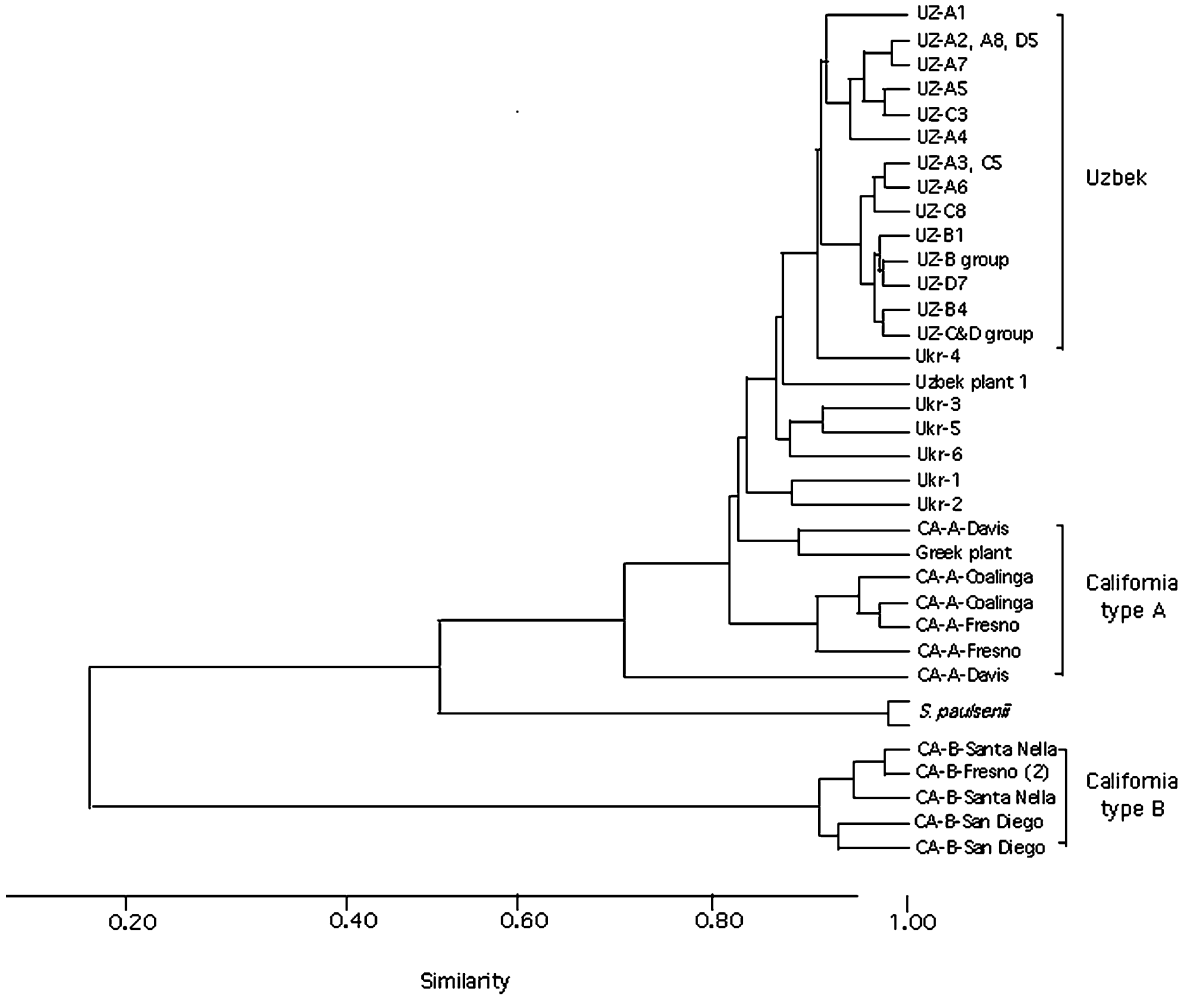

Fig. 3. A dendrogram showing relationships among *S. tragus* from Uzbekistan, Greece, and Ukraine; *S tragus* type A and type B from California; and *S. paulsenii.* Accessions from Uzbekistan are denoted UZ; the letters A–D denote the location of collection followed by the plant number. The Uzbek C and D groups contain plants: C1, C2, C4, C6, C7, D1, D2, D3, D4, and D6. The Uzbek B group contains B2, B3, B5, B6, B7, B8, and D8. Uzbek Plant 1 was the piece of type A that was included with the type B plant grown in the field experiment. Ukr-1 to Ukr-3 were plants collected near Kiev, Ukraine, while Ukr-4 to Ukr-6 were from Kherson Province in southern Ukraine (Table 1). The Greek plant was from Kozani, Greece (Table 1). Accessions from California are designated CA, followed by a notation for type, A or B, and an abbreviation for site of origin; see Table 1. Mantel's Z statistic was 0.99.

the RAPD analysis with primer C18. However, individual pieces of Uzbek Plant 1 produced amplification products that were characteristic of *S. tragus* type A or type B, indicating that this sample contained pieces of both types. The simplest explanation is that a native type A plant was growing in close proximity to the transplanted type B and that the branches commingled as the plants developed. The observation that this plant supported 28 galls at the July measurement is in accord with it being type A rather than type B. The position of the type A specimen from this plant in the dendrogram, close to other Uzbek specimens of type A and the specimens from Ukraine indicates that it is different from the California type A.

While understanding the genetic structure of the target weed population is important, investigation of the genetic variability of the gall midge within its native range may also prove useful, because local populations may have different host specificities. Madeira et al. (2001) investigated genetic variability in the maleleuca snout beetle [*Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae)] in its native range in Australia. Beetles from one site had already been tested and released for biological control of maleleuca (*Maleleuca quinquenervia* (Cav.) Blake) in Florida. Genetic differences were found between insects from two sites, and the authors concluded that additional testing of specificity and host range were justified for insects from the second site.

The objective of biological control researchers is to introduce the most effective complex of biocontrol agents, but using the smallest number of different agents. The costs associated with the introduction of each new agent are substantial and the introduction of less effective agents can increase redundancy of attack

and risk to nontarget organisms. The present study demonstrates the benefit of understanding the genetic structure of an invasive plant as part of a search for control agents. Examination of similarities between problem populations and sources of biocontrol agents will greatly improve chances of introducing the most effective control agents, thereby lowering research and implementation costs and reducing the risk to nontarget organisms.

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References

Burdon, J.J., Groves, R.H., Cullen, J.M., 1981. The impact of biological control on the distribution and abundance of *Chondrilla juncea* in south-eastern Austr. J. Appl. Ecol. 18, 957–966.

Burdon, J.J., Marshall, D.R., Groves, R.H., 1980. Isozyme variation in *Chondrilla juncea* L. in Australia. Aust. J. Bot. 28, 193–198.

Colosi, J.C., Schaal, B.A., 1997. Wild proso millet (*Panicum miliaceum*) is genetically variable and distinct from crop varieties of proso millet. Weed Sci. 45, 509–518.

Gassmann, A., Schroeder, D., 1995. The search for effective biological control agents in Europe: history and lessons from leafy spurge (*Euphorbia esula* L.) and cypress spurge (*Euphorbia cyparissias* L.). Biol. Control 5, 466–477.

Harris, P., 1985. Biocontrol of weeds: bureaucrats, botanists, beekeepers and other bottlenecks. In: Delfosse, E.S. (Ed.), Proceedings of the Sixth International Symposium on the Biological Control of Weeds. Agriculture Canada, Ottawa, pp. 3–12.

Hasan, S., 1985. Search in Greece and Turkey for *Puccinia chondrillina* strains suitable to Australian forms of skeleton weed. In: Delfosse, E.S. (Ed.), Proceedings of the Sixth International Symposium on the Biological Control of Weeds. Agriculture Canada, Ottawa, pp. 652–632.

Hasan, S., Chaboudez, P., Espiau, C., 1995. Isozyme patterns and susceptibility of North American forms of *Chondrilla juncea* to European strains of the rust fungus *Puccinia chondrillina*. In: Delfosse, E.S., Scott, R.R. (Eds.), Proceedings of the Eighth International Symposium on the Biological Control of Weeds, DSIR/CSIRO, Melbourne, Australia, pp. 367–373.

Hetherington, S.D., Irwin, J.A.G., 1999. Pathological and molecular genetic variation in the interaction between *Sporobolus* spp. and *Bipolaris* spp. Aust. J. Agric. Res. 50, 583–588.

Lym, R.G., Nissen, S.J., Rowe, M.L., Lee, D.J., Masters, R.A., 1996. Leafy spurge (*Euphorbia esula*) genotype affects gall midge (*Spurgia esulae*) establishment. Weed Sci. 44, 629–633.

Madeira, P.T., Hale, R.E., Center, T.D., Buckingham, G.R., Wineriter, S.A., Purcell, M., 2001. Whether to release *Oxyops vitiosa* from a second Australian site into Florida's melaleuca? A molecular approach. BioControl 46, 511–528.

Mayo, G.M., Roush, R.T., 1997. Genetic variability of *Hypericum perforatum* L. (Clusiaceae) and the detection of resistance to the biological control agent *Aculus hyperici* Liro (Eriophyidae). Plant Prot. Quart. 12, 70–72.

Nissen, S.J., Master, R.A., Rowe, M.L., 1995. DNA-based marker systems to determine genetic diversity of weedy species and their application to biocontrol. Weed Sci. 43, 504–513.

Paltridge, N.G., 2000. Applications for molecular biology in weed management. Plant Prot. Quart. 15, 50–56.

Pyankov, V.I., Artyusheva, E.G., Edwards, G.E., Black, C.C., Soltis, P.S., 2001. Phylogenetic analysis of tribe Salsolae (Chenopodiaceae) based on ribosomal ITS sequences: implications for the evolution of photosynthesis types. Am. J. Bot. 88, 1189–1198.

Rilke, S., 1999. Revision der Sektion *Salsola* s.l. der Gattung *Salsola* (Chenopodiaceae). Bibl. Bot. (Stuttgart) 149, 1–190.

Rohlf, F.J., 1997. NTSYSpc. Numerical Taxonomy and Multivariate Analysis System, Version 2.02. Exeter Software, Setauket, New York.

Rosen, D., 1986. The role of taxonomy in effective biocontrol programs. Agric. Ecosyst. Environ. 15, 121–129.

Rowe, M.L., Lee, D.J., Nissen, S.J., Bowditch, B.M., Masters, R.A., 1997. Genetic variation in North American leafy spurge (*Euphorbia esula*) determined by DNA markers. Weed Sci. 45, 446–454.

Ryan, F.J., Ayres, D.R., 2000. Molecular markers indicate two cryptic, genetically divergent populations of Russian thistle (*Salsola tragus*) in California. Can. J. Bot. 78, 59–67.

Ryan, F.J., Bell, D., Sobhian, R., 2002. What's in a name? Diversity and origin of Russian thistle in California through DNA markers. In: Hoddle, M.S. (Ed.), California Conference on Biological Control. University of California, Riverside, pp. 51–56.

SAS Institute, Inc., 1996. The SAS System for Windows: Release 6.12. SAS Institute, Inc., Cary, NC.

Scott, L.J., Lange, C.L., Graham, G.C., Yeates, D.K., 1998a. Genetic diversity and origin of siam weed (*Chromolaena odorata*) in Australia. Weed Technol. 12, 27–31.

Scott, L.J., Congdon, B.C., Playford, J., 1998b. Molecular evidence that fireweed (*Senecio madagascariensis*, Asteraceae) is of South American origin. Plant. Syst. Evol. 213, 251–257.

Sobhian, R., Andres, L.A., 1978. The response of skeletonweed to *Cystiphora schmidti* and the gall mite *Aceria chondrillae*. Environ. Entomol. 7, 506–508.

Vaughn, L.K., Ottis, B.V., Prazak-Havey, A.M., Bormanns, C.A., Sneller, C., Chandler, J.M., Park, W.D., 2001. Is red rice found in commercial rice really *Oryza sativa*? Weed Sci. 49, 468–476.

Wetzel, D.K., Horak, M.J., Skinner, D.Z., 1999. Use of PCR-based molecular markers to identify weedy *Amaranthus* species. Weed Sci. 47, 518–523.